(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
17 October 2002 (17.10.2002)

PCT

(10) International Publication Number
WO 02/081785 A1

(51) International Patent Classification[7]: C30B 7/00, 29/58

(21) International Application Number: PCT/GB02/01567

(22) International Filing Date: 2 April 2002 (02.04.2002)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
0108289.0 3 April 2001 (03.04.2001) GB

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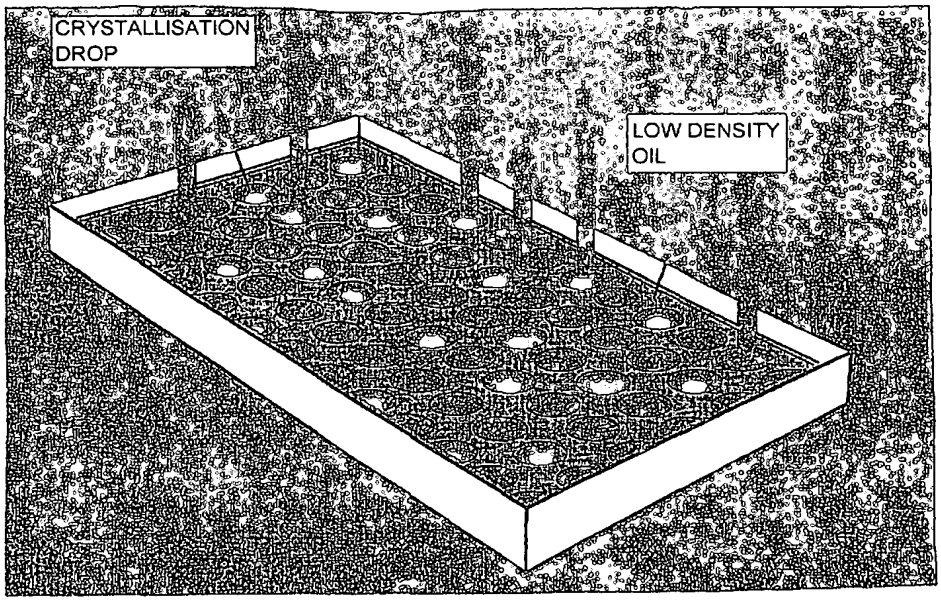
(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report*

[Continued on next page]

(54) Title: CRYSTAL OPTIMISATION TECHNIQUE



(57) Abstract: The present invention relates to a method of producing macromolecular crystals comprising the steps (i) dispensing a solution of macromolecule and crystallising agent so that the solution is or becomes under a layer of oil, wherein the oil layer is such that evaporation from the solution is permitted; (ii) incubating the solution for at least 1 minute; and (iii) subsequently administering additional oil to the oil layer such that evaporation from the solution is decreased, and materials useful in said method.

WO 02/081785 A1

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

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CRYSTAL OPTIMISATION TECHNIQUE

The present invention relates to a technique for optimising crystallisation of macromolecules and its application to automated and high throughput systems.

Automation is crucial for high throughput crystallisation as well as for the other phases of structural genomics since the search for good crystals requires the testing of many different crystallisation conditions. In the area of crystallisation, the main effort and resources have been invested into the automation of screening procedures to identify crystallisation conditions. However, in spite of the ability to generate numerous trials, so far only a small percentage of the proteins produced have led to structure determinations. This is because screening in itself is not usually enough; it has to be complemented by an equally important procedure in crystal production, namely crystal optimisation.

The subject of protein crystallisation has recently gained a new strategic relevance in the next phase of the genome project in which X-ray crystallography will play a major role. There have been major advances in the automation of protein preparation and also in the X-ray analysis and bio-informatics stages once diffraction quality crystals are available. But these advances have not yet been matched by equally good methods for the crystallisation process itself.

Optimisation techniques have been somewhat neglected, mainly because it was mistakenly hoped that large-scale screening would alone produce the desired results. In addition, optimisation has relied on particular individual methods that are often difficult to automate and to adapt to high throughput.

The real stumbling block in structural genomics has become apparent from various pilot projects which are currently under way. These show that the success rate of getting from cloned protein to structure determination is only about 5-10%. For example, figures taken from the Human Proteome Structural Genomics pilot project [Brookhaven National Laboratory, The Rockefeller University and Albert Einstein College of Medicine: http://proteome.bnl.gov/progress.html] show that, out of 123 proteins which were cloned, 59 were purified. Of these 59, 33 yielded some crystals of some sort after routine screening. However, only 15 of these crystals were of good enough quality to be useful for structure determination and only 10 have been solved to date (Fig. 1). Similar success rates by other structural genomics projects from around the world have been reported at the structural genomics conference in Cambridge, September 2000: http://www.mgms.org/cambs2000. Clearly this is highlighting a general problem where, even if proteins can be cloned, expressed, solubilised and purified, and even if crystallisation trials do yield some crystals, this usually does not guarantee that the crystals will be good enough for the structure to be solved. For structural genomics to be productive, it is essential that this problem is addressed.

Recently major advances have been made both in the automation of protein expression and purification methods [1] and in X-ray analysis [2] and modelling [3]. In the case of crystallisation, now that commercial screening kits and computer algorithms for designing arrays of potential conditions are readily accessible, it is no longer a major problem to dispense trials automatically [4,5]. Automatic generation of high throughput screening crystallisation trials is also underway [2,6]. However, there are a number of issues that still require attention. These include: (i) the large amount of manual preparation needed prior to automated dispensing, (ii) the issue of cleaning hundreds of syringes and (iii) the viewing, follow-up and analysis

of the results. Some proteins will surely crystallise during this initial screening, but most trials are likely to yield microcrystals or low-ordered crystals. The conversion of such crystals into useful ones requires intellectual input and individualised optimisation techniques. Such techniques do not lend themselves readily to automation and they have yet to be adapted to cope with the huge volume of experiments required by Genome Projects. Consequently, the subject of optimisation has been rather neglected, apart from the obvious first step of merely changing the concentrations or pH around the conditions of interest. The need for high-throughput at every stage in structural genomics prompted us to start to design automated optimisation methods which go beyond the usual fine-tuning of conditions.

Manual optimisation methods are known, some of which have resulted in successful crystallisation of proteins that could not be crystallised otherwise [e.g. 7,8]. We have now developed automated optimisation techniques.

A first aspect of the invention provides a method of producing macromolecular crystals comprising the steps

(i) dispensing a solution of macromolecule and crystallising agent so that the solution is or becomes under a layer of oil, wherein the oil layer is such that evaporation from the solution is permitted;

(ii) incubating the solution for at least 1 minute; and

(iii) subsequently administering additional oil to the oil layer such that evaporation from the solution is rendered negligible.

The macromolecule may be any macromolecule, but it is preferred if it is a biological macromolecule. It is still more preferred if the biological macromolecule is a polypeptide.

The crystallisation agent and macromolecule may be dispensed under the oil at the same time, or may be dispensed separately or sequentially in any order.

Crystallisation agents are known in the art and the composition may be optimised according to the nature of the macromolecule to be crystallised. Typical crystallisation agents include salt and buffer.

The rate of evaporation of solvent from the drop of sample or solution under the oil may be decreased such that it is less than before addition of the oil in step (iii), or it may be decreased to the extent that it is negligible.

By "negligible" we mean the evaporation from the sample drop under the oil is undetectable over a period of at least a day, preferably over a period of at least 2 days, or 5 days or a week. Preferably, negligible evaporation is a loss of water from a solution which is sufficiently small that it cannot be detected after a period of at least two weeks or 1 month or 2 months or 3 months. Evaporation from the sample drop may be judged by any suitable means, including by assessment of the size of the sample drop, or by the appearance of dryness.

The oil may be any suitable liquid oil. Advantageously, the oil is of a density lower than that of the liquid to be dispensed through the tip capable of dispensing a volume between 0.1µl and 1ml. This is because the oil is preferably one which is used to overlay a crystallisation drop. If the oil were denser than the liquid in the crystallisation drop, it would fail to "sit"

on top of the drop. Preferably, the oil has a density of around 0.84 g cm^{-3}. Furthermore it is preferred if the oil dispensed by the system is one which can act as an inert sealant and does not interact with crystallisation trials, for example, one that does not cause precipitation. Hence, it is preferred if the oil consists of or comprises paraffin. More preferably the oil consists of paraffin light, a purified mixture of liquid saturated hydrocarbons obtained from petroleum.

According to one embodiment of this aspect of the invention, the oil added in step (iii) is sufficient to slow the evaporation from the solution but does not render evaporation negligible, and the method is supplemented with a further step, step (iv), in which yet more oil is added to the oil layer, in an amount or type which renders evaporation from the solution negligible.

By adding oil first in an amount which decreases evaporation but does not halt it, the evaporation may be slowed gradually before stopping. This permits a further level of control over the speed or pattern of solvent evaporation.

The biological macromolecule may be any biological macromolecule including nucleic acids, complex polysaccharides and viruses. Preferably, the biological macromolecules are polypeptides. A polypeptide comprises at least one chain of amino acid residues which are covalently joined by peptides bonds. A polypeptide chain may have any number of amino acid residues, preferably at least two, more preferably at least 100, 500, 1000 or 2000. The polypeptide chain may have more than 2000 residues. A polypeptide may contain residues in the chain which are unusual or artificial, and may comprise non-peptide bonds such as disulphide bonds. The residues may be further modified, for example to include a phosphate group or a sugar chain (eg an oligosaccharide) or a lipid moiety. A

polypeptide may comprise more than one chain (for example, two chains linked by a disulphide bond between the sulphur in the side chain of cysteine residues), and may further comprise inorganic or organic co-factors or groups. Such modifications and additions are included within the term "polypeptide".

Preferably, the concentration of the biological macromolecule solution under the oil of step (i) is undersaturated or metastable. In other words, the concentration of the solution is outside the nucleation zone of the phase diagram of that solution.

It is further preferred if the oil in step (iii) is only added once the concentration of the biological macromolecule solution reaches the nucleation zone of the solution phase diagram. An example of a crystallisation phase diagram is shown in Chayen *et al* (1996) *Q Reviews of Biophysics* 29:227-278.

Advantageously, the oil layer of step (i) allows evaporation from the solution of biological macromolecule because the thickness of the oil layer is insufficient to render the evaporation negligible. Typically, the thickness of the layer of oil at which evaporation ceases to be negligible is less than 3.5mm. Preferably, the thickness of the layer of oil is between 0.7 to 1.2mm.

It will be appreciated that the oil layer of step (i) may be a single oil type or may comprise a mixture of at least two oils. Preferably the oil layer is a single oil and not a mixture, since this provides the advantage of making the evaporation kinetics easier to judge, and allows for a complete block of evaporation from the sample (ie, where evaporation of solvent is negligible). A suitable oil for the layer of step (i) includes paraffin. It is

preferred if the oil layer consists of paraffin, and preferably the paraffin is one such as is available from Hampton Research, CA 92677-3913 USA under catalogue no HR3-411. Where a mixture of oils is used, it is preferred if it is a mixture of paraffin and silicone. A suitable mixture of paraffin and silicone is 65% paraffin and 35% silicone. The use of oil and mixtures of oil to permit diffusion through the layer is described in D'Arcy *et al.* (1996) *J. Crystal Growth* **168**, 175-180 and Chayen (1997) *J. Appl. Cryst.* 30:198-202.

The thickness of the oil layer which permits evaporation from the underlying solution of biological macromolecule may vary according to the composition of the oil layer. Hence, the type of oil and the thickness of the oil layer will dictate the rate of evaporation and consequently the speed at which the nucleation zone is approached. A person skilled in the art may easily determine the thickness of oil and the composition of oil layer in step (i) which is most suitable for the desired rate of solvent evaporation from the sample or solution of biological macromolecule.

The oil of step (ii) may be administered by any suitable means but it is preferred if it is administered by an automated liquid dispensing system. Automated fluid dispensing systems are known, and a suitable example is the IMPAX and Oryx 6 systems produced by Douglas Instruments Ltd (Douglas House, Hungerford, Berks UK). Preferably, a system such as the IMPAX machine is modified such that it comprises at least one further arm with a tip that is capable of dispensing volumes of oil between 10µl and 1ml. More preferably, the system is the Oryx 6 system.

According to a preferred embodiment of this aspect of the invention, the point at which the additional oil of step (iii) is added, ie, the length of the

incubation of step (ii) is determined by an initial screen. Briefly, a suitable initial screen would involve setting up a crystallisation trial (ie, a series of crystallisation samples) where the oil layer is one which permits evaporation from the trial sample. The appearance of crystals would be monitored for at time intervals of, for example in an overnight screen, 2, 4, 6, and 12 hours after setting up the trial. Where crystals are not formed in such an initial screen, or it is not expected that crystals would form within 24 hours after setting up the samples, the appearance of crystals could be monitored for every 24 hours after the samples were set up. It known in the art that the stage at which crystals form is related to both nucleation kinetics and osmotic parameters, and a person skilled in the art of crystallography can consider these parameters when establishing a screen or optimisation trial. The appearance of crystals may be determined by any convenient means known in the art of crystallography. For example, crystals may be observed by eye, or by using a light scatterometer as described by Rosenberger *et al* (1993) *J. Crystal Growth* **129**:1-12. Where a light scatterometer is used, scintillation from micron size crystals causes a distinct increase in the photo diode signal from the light scattering cell, and it is this increase which indicates crystal formation.

The time taken for crystals of a particular macromolecule to be detectable in the initial screen is the incubation time of step (ii), and following this time is the point at which further oil may be added in step (iii) to reduce evaporation from the crystallisation sample, preferably to a negligible level, and thereby promote crystal growth (as opposed to nucleation).

A second aspect of the invention provides a use of an automated liquid dispensing system in the method of the first aspect of the invention.

The automated liquid dispensing system may be any suitable dispensing system, including the IMPAX and Oryx 6 systems available from Douglas Instruments, Hungerford, Berks, UK, provided it is capable of dispensing oil. Preferably the dispensing system is capable of being programmed to dispense further oil on the crystallisation sample at a determined timepoint or range of timepoints.

A third aspect of the invention provides a use of oil to decrease or halt evaporation from a solution of a macromolecule which solution is under a layer of oil which oil layer is such that it allows evaporation from the underlying solution.

Thus, according to this aspect, the oil is added to oil which is already present on the solution. The solution is preferably one from which there has already been some evaporation of solvent, and the oil addition according to this aspect of the invention is to decrease that evaporation, or halt it so that it is negligible. In other words, there is a delay of at least 1 minute, preferably 10 minutes, more preferably at least 30 minutes or 1 hour between the first application of oil (which is such that it allows evaporation from the underlying solution) and the subsequent application of oil according to this aspect of the invention.

For the avoidance of doubt, the use of oil according to this aspect is not a use for preventing evaporation from a sample where oil has not previously been layered on, or contacted with the sample, or has been applied to or contacted with the sample in less than 1 minute previously, or less than 5 or 10 or 30 minutes previously.

Any oil which is capable of halting evaporation from a solution of macromolecule may be used. Preferably, the oil is paraffin.

Preferably, the paraffin is used to halt evaporation from the solution of macromolecule rather than just reduce or decrease evaporation. By "halt" we mean that evaporation from the solution is negligible, as defined above.

Preferably the solution of macromolecule is a solution of a biological macromolecule. More preferably, the solution is a solution of protein. Preferable the solution is a sample which further comprises crystallisation agents.

It is further preferred if the use of the paraffin oil is at a stage where nucleation and/or crystallisation of the macromolecule in the solution or sample has begun.

A fourth aspect of the invention provides a use of paraffin oil to permit evaporation from a solution of a macromolecule which solution is under a layer of the paraffin which paraffin layer is sufficiently thin that it allows evaporation from the underlying solution.

The paraffin oil may be present as one component in a mixture of oils. A suitable second component in such a mixture includes silicon oil. Preferably the paraffin is added as the only oil over the solution of macromolecule and is not used as a mixture with another oil.

A thickness of paraffin which permits evaporation from an underlying solution or sample may be determined by a person skilled in the art using only routine experimentation, and may vary according to the desired rate of evaporation from the sample or solution. Typically, for a crystallisation sample, the thickness which permits a useful rate of evaporation from the sample is between 0.7-1.2mm, which corresponds to 0.25ml-0.5ml of oil

over a sample of between 0.7µl - 2µl in a microbatch plate measuring 5.5 x 8 x 0.9cm. As is known in the art, an oil thickness of 4mm or more renders evaporation from an underlying sample or solution negligible.

The invention will now be described in more detail with the aid of the following Figures and Examples:

Figure Legends

Figure 1. The steps leading to X-ray structure determination and their success rates. Histogram showing the success rate of the different stages from clone to structure, taken from a progress report of a structural genomics project which is publicly available on the Internet (see text). Getting good crystals from purified protein is the main block to rapid progress.

Figure 2. Controlled evaporation.

(a) Microbatch drops under a thin layer of oil which allows their concentration (symbolised by the arrows).

(b) Arrest of evaporation/ concentration by topping up the oil to produce a thicker layer above the drops. From then onwards, the experiment follows its 'conventional' route.

Example 1.

Promotion of nucleation and subsequently arresting nucleation using oils in microbatch.

An initial screen was set up with drops of lysozyme in a tray, for example, drops containing final concentrations of 20mg/ml of lysozyme in 50 mM

sodium acetate buffer pH 4.7 and 6% NaCl were dispensed using the IMPAX and put under a thin layer (0.7mm) of oil. The tray was set aside and checked for when crystals appeared. If the first crystals appeared after, for example, 8 hours, several other trays were set up at the same conditions and oil added (to more than 3.5 mm thick) to each tray at different time intervals. The time intervals were such that one tray was covered with further oil to arrest nucleation by decreasing evaporation after half an hour, another tray after 1 hour, then another after 2, 4 and 6 hours etc. It was anticipated that one of these times would give the best result in terms of crystal formation. In the case of lysozyme, the time was 3 hours. In other words, the tray was set up with the drops of macromolecule to be crystallised (1-2 microlitres) and covered with the thin oil layer, then arrested by adding more oil after 3 hours.

Where crystals take 1 week to grow, the addition of further oil to arrest nucleation by decreasing evaporation to a negligible level is done at intervals of 12- 24 hours.

To remove the requirement for trial and error, the time point when the nucleation takes place can be determined by monitoring the drops by light scattering as done by Rosenberger *et al* (1993) *J. Crystal Growth* **129**, 1-12.

The first semi-high-throughput experiments for both screening and optimisation were designed in 1990 as microbatch trials under oil [4]. Microbatch trials consisting of 0.7 – 2 µl drops of a mixture of protein and crystallising agents are generated by an automated system called IMPAX and are dispensed and incubated under oil in order to prevent evaporation. The IMPAX system has two modes of action: one is used to automatically screen numerous potential crystallisation conditions and the other is used for optimisation of the most promising screening conditions using a matrix

survey [9,10]. The microbatch method has established a unique way of crystallising macromolecules, and many target proteins have been successfully crystallised using it [e.g. 11,12,13]. In its current state, a single IMPAX machine can generate about 2000 trials per day. Because batch is mechanically the simplest crystallisation method, this procedure lends itself for adaptation to high-throughput crystallisation. The microbatch method has already been adapted for high throughput screening experiments in the USA using a large bank of syringes dispensing 0.4 – 0.5 µl volumes into 1536-well micro-assay plates [6].

Control of evaporation kinetics

It is well known that nucleation is a pre-requisite for and the first step in crystal growth, yet excess nucleation yields a large number of small crystals instead of a small number of useful ones. A means of controlling nucleation by reaching nucleation slowly and then stopping it before it becomes excessive can now be carried out in microbatch. This is achieved by controlled evaporation, and therefore concentration, of the drops through a thin oil layer. Evaporation is later arrested by increasing the thickness of the oil layer.

The paraffin oil generally used in standard microbatch trials is not completely impermeable to the aqueous solution which constitutes the crystallisation drops. The conventional microbatch method involves using a layer of oil thick enough to render evaporation through it negligible within the time scale of the experiment (4 mm, corresponding to ca. 8 ml in a typical crystallisation microbatch plate measuring 5.5 x 8 x 0.9 cm). However, if controlled evaporation is required, the thickness of the layer can become an active parameter of the process. Instead of setting the microbatch conditions well inside the nucleation zone of the phase diagram,

conditions are set to be undersaturated or metastable and water is allowed to evaporate slowly through a thin oil layer (0.7-1.2 mm, corresponding to 0.25-0.5 ml; Fig. 2(a)). The solution therefore arrives at the nucleation zone in a controlled way. The thickness of the oil layer is then increased, rendering evaporation negligible, and the experiment progresses along the conventional batch route (Fig. 2(b)). The critical incubation time at which to add further oil to give conditions suitable for optimal crystal production depends on the system and is obviously related to both nucleation kinetics and osmotic parameters. This procedure can be conducted automatically with an extra arm added to the IMPAX machine with which to dispense oil after set time intervals.

So far this procedure has been tried with three model proteins: trypsin, lysozyme, and a cyanin protein. In all cases an enhancement of the average size and of the yield of useful diffraction quality crystals was achieved compared with results from the standard microbatch method. The standard method resulted in showers of microcrystals in many of the drops, something which was almost completely avoided when applying the controlled evaporation technique.

Slowing down vapour diffusion with an oil barrier

A means to slow down the equilibration rate and thereby approach supersaturation more slowly in order to avoid crystal "showers" is by placing a paraffin/ silicone oil mixture as a barrier over the reservoir of a hanging or sitting drop trial [23]. The mix of paraffin and silicone oil can be varied as needed. It was found that volumes of 250-500 µl placed over 1ml reservoirs in standard Linbro plates (corresponding to layer thickness of 1.25 - 2.5 mm) were most efficient. This method has been shown to work well for several proteins [e.g. 7,19]. The advantage of this technique is that

no change is required to the crystallisation conditions nor to the method used. It can be applied in Linbro, VDX, Cryschem or any other vessel, and it can easily be automated by adding an extra step to the procedure used by robots such as the Cyberlab.

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CLAIMS

1. A method of producing macromolecular crystals comprising the steps
   (i) dispensing a solution of macromolecule and crystallising agent so that the solution is or becomes under a layer of oil, wherein the oil layer is such that evaporation from the solution is permitted;
   (ii) incubating the solution for at least 1 minute; and
   (iii) subsequently administering additional oil to the oil layer such that evaporation from the solution is decreased.

2. A method according to Claim 1 wherein the evaporation from the solution is rendered negligible.

3. A method according to Claims 1 or 2 wherein the concentration of the macromolecule solution under the oil of step (i) is undersaturated or metastable.

4. A method according to any one of Claims 1 to 3 wherein the oil in step (ii) is only added once the concentration of the macromolecule solution reaches the nucleation zone of the solution phase diagram.

5. A method according to any one of Claims 1 to 4 wherein the thickness of the layer of oil is less than 3.5mm.

6. A method according to Claim 5 wherein the thickness of the layer of oil is between 0.7 to 1.2mm.

7. A method according to any one of Claims 1 to 6 wherein the oil is paraffin oil.

8. A method according to any one of Claims 1 to 7 wherein the oil of step (ii) is administered by an automated liquid dispensing system.

9. Use of an automated liquid dispensing system in the method of any one of Claims 1 to 8.

10. Use according to Claim 9 wherein the automated liquid dispensing system is an Oryx 6 or IMPAX system.

11. Use of oil to decrease or halt evaporation from a solution of a macromolecule which solution is under a layer of oil which oil layer is such that it allows evaporation from the underlying solution.

12. Use according to Claim 11 wherein the oil halts evaporation from the solution.

13. Use of oil to permit evaporation from a solution of a macromolecule which solution is under a layer of the oil which oil layer is sufficiently thin that it allows evaporation from the underlying solution.

14. Use according to any one of Claims 10 to 13 wherein the oil is paraffin oil.

15. Use according to Claim 14 wherein the paraffin is present as one component in a mixture of oils.

16. A method according to any one of Claims 1 to 8 or a use according to any one of Claims 10 to 15 wherein the macromolecule is a biological macromolecule.

17. A method or use according to Claim 16 wherein the biological macromolecule is a polypeptide.

FIGURE 1

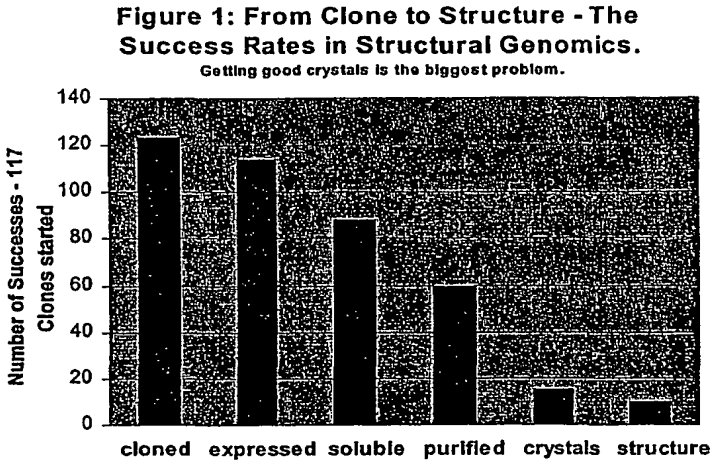
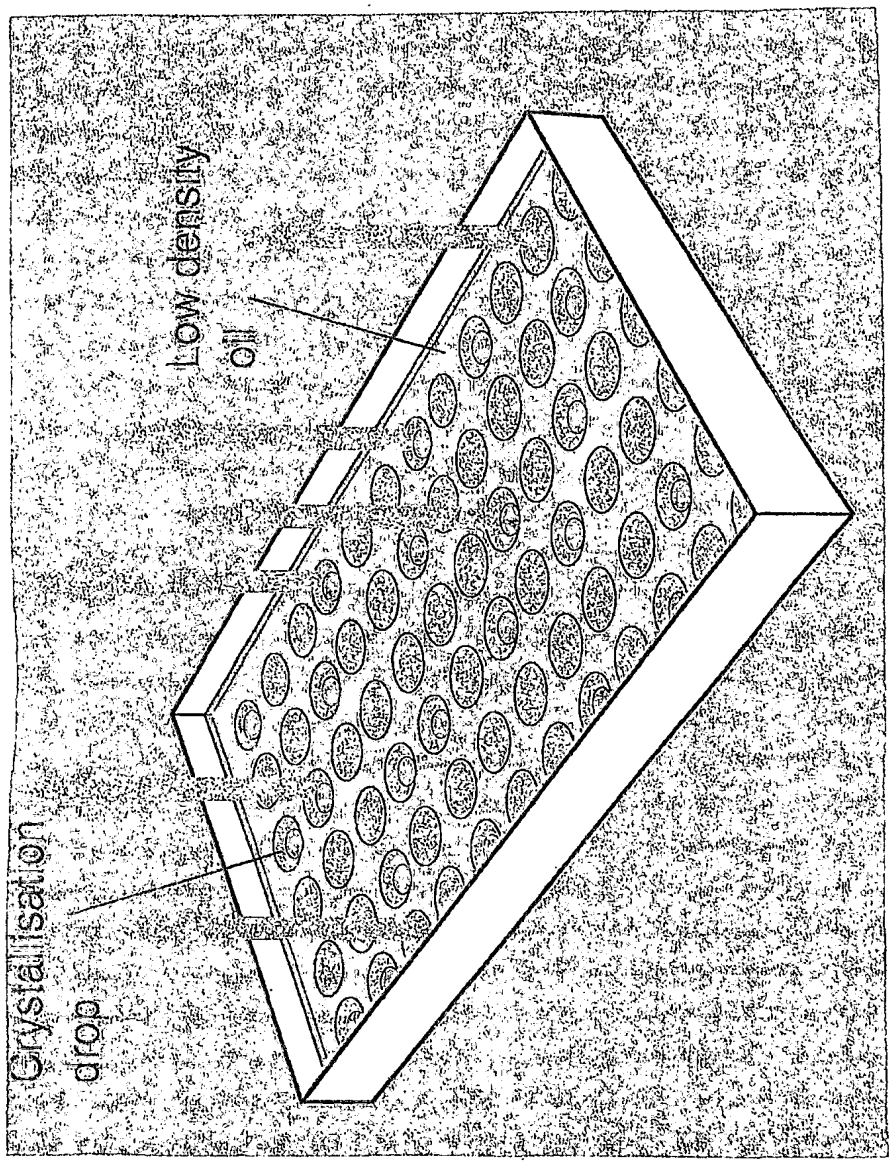


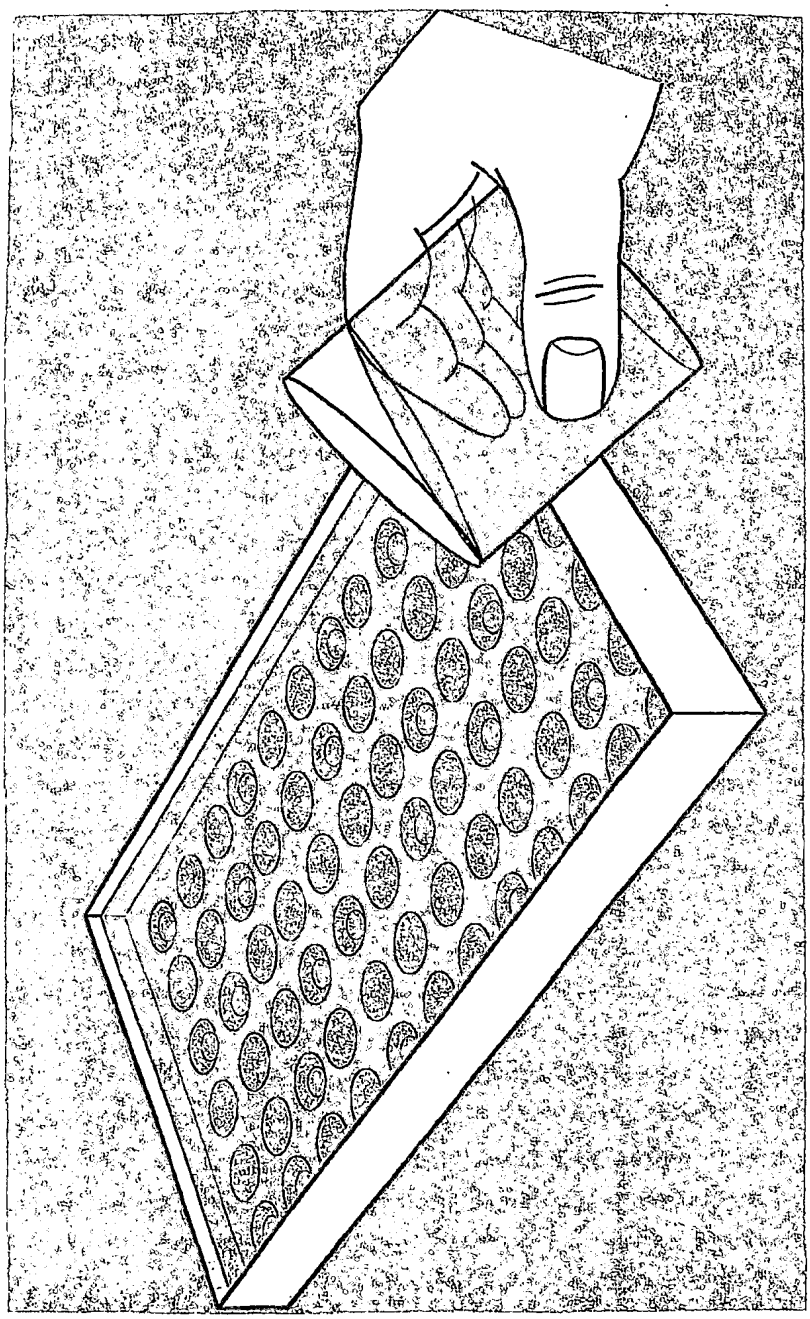
Figure 1: From Clone to Structure - The Success Rates in Structural Genomics.
Getting good crystals is the biggest problem.
Number of Successes - 117 Clones started
140
120
100
80
60
40
20
0
cloned
expressed
soluble
purified
crystals
structure

FIGURE 2



(a)

FIGURE 2



(b)

INTERNATIONAL SEARCH REPORT

national Application No
PCT/GB 02/01567

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C30B7/00 C30B29/58

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C30B

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, PAJ, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	CHAYEN: "The role of oil in macromolecualr crystallization" STRUCTURE, vol. 5, no. 10, 15 October 1997 (1997-10-15), pages 1269-1274, XP008005793 cited in the application page 1270, right-hand column --- -/--	11-15

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 July 2002	12/08/2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Cook, S

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	CHAYEN N E: "Crystallization with oils: a new dimension in macromolecular crystal growth" JOURNAL OF CRYSTAL GROWTH, NORTH-HOLLAND PUBLISHING CO. AMSTERDAM, NL, vol. 196, no. 2-4, 15 January 1999 (1999-01-15), pages 434-441, XP004154378 ISSN: 0022-0248 page 653, right-hand column	1,7,16, 17
A	CHAYEN N E: "Recent advances in methodology for the crystallization of biological macromolecules" JOURNAL OF CRYSTAL GROWTH, NORTH-HOLLAND PUBLISHING CO. AMSTERDAM, NL, vol. 198-199, March 1999 (1999-03), pages 649-655, XP004170934 ISSN: 0022-0248 page 435	1
A	CHAYEN N E ET AL: "MICROBATCH CRYSTALLIZATION UNDER OIL - A NEW TECHNIQUE ALLOWING MANY SMALL-VOLUME CRYSTALLIZATION TRIALS" JOURNAL OF CRYSTAL GROWTH, NORTH-HOLLAND PUBLISHING CO. AMSTERDAM, NL, vol. 122, no. 1 / 4, 2 August 1992 (1992-08-02), pages 176-180, XP000306492 ISSN: 0022-0248 cited in the application the whole document	1
A	CHAYEN N E ET AL: "AN AUTOMATED SYSTEM FOR MICRO-BATCH PROTEIN CRYSTALLIZATION AND SCREENING" JOURNAL OF APPLIED CRYSTALLOGRAPHY, COPENHAGEN, DK, vol. 23, 1990, pages 297-302, XP000213540 ISSN: 0021-8898 cited in the application page 298	1,8,9
A	GB 2 249 492 A (DOUGLAS INSTR LTD ;IMPERIAL COLLEGE (GB)) 13 May 1992 (1992-05-13) the whole document	1,9-17
A	WO 00 78445 A (JAKLEVIC JOSEPH M ;SANTARSIERO BERNARD D (US); STEVENS RAYMOND C () 28 December 2000 (2000-12-28)	

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INTERNATIONAL SEARCH REPORT

national Application No
PCT/GB 02/01567

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	STEWART P S ET AL: "A comparison of microbatch and vapour diffusion for initial screening of crystallization conditions" JOURNAL OF CRYSTAL GROWTH, NORTH-HOLLAND PUBLISHING CO. AMSTERDAM, NL, vol. 168, no. 1, 1 October 1996 (1996-10-01), pages 170-174, XP004013784 ISSN: 0022-0248	
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			EP	1204467 A1	15-05-2002
			WO	0078445 A1	28-12-2000